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Oct-4 Regulates Alternative Platelet-derived Growth Factor α Receptor Gene Promoter in Human Embryonal Carcinoma Cells*

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Expression of the platelet-derived growth factor α-receptor (PDGFαR) gene is tightly controlled in mammalian embryogenesis. A well established model system to study human embryogenesis is the embryonal carcinoma cell line Tera2. We have shown previously that retinoic acid-differentiated Tera2 cells express two PDGFαR transcripts of 6.4 kilobase pairs (kb) (encoding the full-length receptor) and 3.0 kb, respectively, whereas in contrast, undifferentiated Tera2 cells express PDFGαR transcripts of 1.5 kb and 5.0 kb. Here we show that this switch in PDGFαR expression pattern during differentiation of Tera2 cells results from alternative promoter use. In undifferentiated cells, a second promoter is used, which is located in intron 12 of the PDGFαR gene. Functional analysis shows that this promoter contains a consensus octamer motif, which can be bound by the POU domain transcription factor Oct-4. Oct-4 is expressed in undifferentiated Tera2 cells but not in retinoic acid-induced differentiated cells. Mutation of the octamer motif decreases promoter activity, while ectopic expression of Oct-4 in differentiated Tera2 cells specifically enhances the activity of this PDGFαR promoter. Therefore, we suggest that an important aspect in the maintenance of the undifferentiated state of human embryonal carcinoma cells results from Oct-4 expression, which thereupon activates this PDGFαR promoter.

Platelet-derived growth factor (PDGF)[1] and its receptors play a prominent role during early mammalian development. Already in the preimplantation embryo of the mouse, from the two-cell stage onwards to the blastocyst stage, the PDGF-A chain is expressed (1), while both this gene and the cognate PDGF α-receptor (PDGFαR) gene are expressed in early postimplantation embryos (2). Murine embryonal carcinoma (EC) cells in culture secrete PDGF-AA (3) and express the PDGFαR following differentiation by retinoic acid (4). The importance of the PDGFαR in mammalian development is also exemplified by the *Patch* (*Ph*) mouse mutant. The *Ph* mutant lacks part of the PDGFαR gene (5, 6) and displays severe developmental defects in mesodermal and neuroectodermal tissues, often resulting in prenatal lethality (7, 8).

An important model system for studying human early embryogenesis is that of testicular germ cell tumors. These tumors are derived from a derangement of a primordial germ cell in early life, which first develops into a noninvasive carcinoma-*in situ* and subsequently grow out as a seminoma or a nonseminomatous tumor (9). The stem cells of nonseminomatous tumors, also referred to as EC cells, strongly resemble cells of the early preimplantation human embryo. Various established human EC cell lines, among others the Tera2 cell line, can be induced to differentiate *in vitro* into a variety of mature, nontumorigenic cell types by the morphogen retinoic acid (10). We have recently shown that differentiation of Tera2 EC cells by retinoic acid (RA) is accompanied by a shift in expression of PDGFαR mRNA variants (11).[2] Four human PDGFαR transcripts have been identified as a result of a combination of alternative splicing and promoter use. Two PDGFαR mRNA species of 1.5 and 5.0 kb, respectively, are expressed in early human embryonic cells, including the undifferentiated Tera2 EC cells. Studies on surgically removed testicular germ cell tumors have shown that the 1.5-kb PDGFαR transcript can be used as a selective marker for carcinoma-*in situ*, seminoma, and undifferentiated nonseminomatous tumors in the human testis.[2] In differentiated cells, including RA-differentiated Tera2 (Tera2 RA) cells, two other PDGFαR transcripts of 6.4 kb, which encodes the functional full-length receptor, and of 3.0-kb, which potentially encodes a dominant negative isoform, have been identified. Aberrant expression of the full-length PDGFαR receptor, encoded by the 6.4-kb transcript, has also been implicated in tumorigenesis, *i.e.* it is overexpressed in various tumors, including gliomas (12).

In a previous study we cloned and characterized the human PDGFαR gene promoter (P1), which gives rise to the 6.4- and 3.0-kb transcripts. Activity of this P1 promoter can be stimulated strongly by RA and cAMP (13). Similar studies with respect to the mouse and rat PDGFαR promoter have been published recently (14, 15). In the present study we have cloned and characterized the second PDGFαR gene promoter (P2), which gives rise to the 1.5- and 5.0-kb transcripts in early embryonic cells. We show here that the P2 promoter, located in intron 12 of the PDGFαR gene, is active in undifferentiated Tera2 cells and is controlled by the POU domain transcription factor Oct-4. Oct-4 expression is detected in Tera2 EC cells but not in Tera2 RA cells.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X95095.

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[1] The abbreviations used are: PDGF, platelet-derived growth factor; PDGFαR and PDGFβR, platelet-derived growth factor-α and -β receptor, respectively; RA, retinoic acid; EC, embryonal carcinoma; kb, kilobase pair(s); kFGF, Kaposi's fibroblast growth factor; EMSA, electrophoretic mobility shift assay.

[2] Mosselman, S., Looijenga, L. H. J., Gillis, A. J. M., van Rooijen, M. A., Kraft, H. J., Van Zoelen, E. J. J., and Oosterhuis, J. W. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 2884–2888.

MATERIALS AND METHODS

Cell Culture—Tera2 clone 13 (Tera2) cells were grown in α-modification of minimal essential medium lacking nucleosides and deoxynucleosides, supplemented with 10% (v/v) fetal calf serum and 44 mM $NaHCO_3$ in a 7.5% CO_2 atmosphere at 37 °C. Differentiation of cells was induced by the addition of RA (5 μM) 16 h after the cells were seeded at low density (5.0×10^3 cells/cm^2) and maintained at this medium for 7 days, prior to further analysis or transfection.

PDGFαR P2 Promoter Constructs—Nucleotide sequence analysis was performed using the Pharmacia T7 sequencing kit. PDGFαR P2 promoter constructs were generated by standard cloning procedures (16), using either restriction fragments or DNA fragments obtained by the polymerase chain reaction. Mutation of the octamer binding site was performed with the Altered Sites system kit (Promega), according to the manufacturer's protocol. All fragments were inserted in the multiple cloning site of the pSLA4 luciferase reporter plasmid (13).

Transfection, Luciferase, and β-Galactosidase Assays—Promoter-luciferase constructs were transiently transfected into either undifferentiated (Tera2 EC) or differentiated (Tera2 RA) cells using the calcium phosphate coprecipitation method (16). Luciferase activity was determined 48 h (Tera2 EC) or 72 h (Tera2 RA) post-transfection (Luciferase assay kit, Promega). The luciferase activity was corrected for transfection efficiency by measuring the β-galactosidase activity of a *lacZ* gene driven by an SV40-promoter of a cotransfected pCH110 plasmid (17). Every experiment was done in duplicate and repeated at least twice with two batches of DNA.

RNA Analysis—Total RNA was isolated from undifferentiated (EC) or differentiated (7 days of RA treatment) Tera2 cells, using the isothiocyanate method (18). After poly(A)$^+$ isolation, the mRNA was quantitated spectrophotometrically and subjected to 1% agarose gel electrophoresis in formamide. The amount and integrity of loaded mRNA was controlled by ethidium bromide staining, after which it was transferred to Hybond-N (Amersham Corp.). Hybridization and washing procedures were carried out as described (11). A mouse Oct-4 cDNA probe (kindly provided by Dr. P. C. van der Vliet, University of Utrecht), was labeled by random priming (19) using a labeling kit (Amersham).

Electrophoretic Mobility Shift Assay (EMSA)—DNA restriction fragments were filled in by Klenow polymerase treatment in the presence of [α-^{32}P]dCTP. Oligonucleotides for EMSAs were end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase. Double-stranded oligonucleotides were separated from single-stranded oligonucleotides by polyacrylamide gel electrophoresis. The oct-1c consensus oligonucleotide was purchased from Promega. Nuclear extracts were prepared as described (20). Binding reactions and gel electrophoresis were performed essentially as described (21). The mouse anti-Oct-4-antibody has been described by Rosfjord and Rizzino (22).

RESULTS

Sequence Determination and Characterization of the P2 Promoter of the PDGFαR Gene—Two alternative PDGFαR transcripts, of 1.5 and 5.0 kb, respectively, are specifically expressed in the undifferentiated Tera2 embryonal carcinoma cells. Although the transcripts terminate differently as a result of alternative splicing, both transcripts initiate in intron 12 of the PDGFαR gene (11).[2] In order to functionally characterize this putative promoter, which was designated P2 promoter, the region was cloned and sequenced (Fig. 1). The sequence upstream from the transcription initiation site lacks a TATA box, which is also the case for the PDGFαR P1 promoter in human (13), mouse (14), and rat (15), and is also not extremely GC-rich. In the promoter region, several consensus binding sites for transcription factors could be detected, including AP1, AP2, and PEA3 motifs (23). A consensus octamer binding site is located in the transcribed region, at positions +28 to +35.

We first set out to determine the functional relevance of these putative binding sites and of other regions required for activity of the PDGFαR promoter. It is of interest to note that the P2 promoter region is demethylated in both undifferentiated and RA-induced differentiated Tera2 cells.[3] A series of progressive deletion mutants of the PDGFαR P2 promoter was

```
-842            t cgagtaatag tagtaatagt agaataggtt tccgtacagc
                                                        PEA3
-801    tggctctgtg tgtaattaaa ccctttttct attgcaattc ccctgtcttg
                                                        PEA3
-751    gtaaatcggc tctgtctagg cggacaagga gaatccatcg ggcggttata
                                                        PEA3
-701    agagctgccc cccaatttca aatatttata tctaagcttt ctttatttcc
                                                        PEA3
-651    ctgcctattt cccaacaagg gatgaggagc ttagggagtt aaaaagtagt
                                                        PEA3
-601    aaaatatgga ggaaaagggc ataattccca ttataccaag aggcattgct
                                                        PEA3
-551    ggtgaaggca ataccttcc aggtacgatt ttcagtaaca cagacgtgcg
                                                        PEA3
-501    agtaagaggc agtgttggct gttagtgtct tttatgagcc agtctttccc
                                                        PEA3
-451    tggcttgcta tccgtggtga gactgacacc caaatgttct ctcagagtct
                                                        PEA3
-401    ctttcagggt ggaacaaaca ggcttcaggg tctttacgta tgtctcctcc
                                                        PEA3
-351    caacatgaag ctaattgctg tgctctcggg catgtttagc tcttggtaga
              PEA3
-301    gtggctttcc taacaaatag ggagcagtgg aggccagcct gaagttttta
          AP1
-251    ttagtcact ccttagaatc gatgatattt tgaatactga agtatttcca
                                                        PEA3
-201    gtggctagta atttactaag acaaaagatg cccctgtttg catatggaaa
                                                        PEA3
-151    acagaagggg agagagccag gaggtgtggg tgagagcccc gaaggcaaga
                AP2
-101    ggatcccagg ggctggccca gcacggagct ggtaagacac ggcgcctcac
                                                        PEA3
 -51    acccagggag ggctgcaccc tcctttctcc cgtctgtgtt ttctttccct
                                                  OCT
   1    TGCAAGTGTT ATTCGACAAA AGCAATTATG CTAATTTCCT TCCTGTGGGC
                                                  PEA3
  51    TCAATTCCTT TTTTTGACAC GATGACTTAG GAGGAGTCAT TATGATTGCT
                                                        PEA3
 101    CCAAACAGGA AAGACACTCG CCCAGCTGTC CGCCCGCAGA GAGCTGGCTA
                                                        PEA3
 151    CGGTGCAGAA AGCTGAGAGG AGGCGTCTGG AGTTTTTGGG TGTTAATGAT
                                                        PEA3
 201    TCTGCCTGCC CACAGGT
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FIG. 1. **The nucleotide sequence of the PDGFαR gene P2 promoter and 5′-untranslated region of the 1.5-kb transcript.** The transcription initiation site (11) is numbered nucleotide 1, and the transcribed region is given in *uppercase letters*. The sequence is given up to exon 13. Indicated in the figure are the consensus binding sites for transcription factors AP1, AP2, and PEA3, which are *underlined*, and for a POU domain transcription factor, which is depicted in *boldface*. Note the absence of a TATA box in the promoter. (GenBank™/EMBL data base accession number X95095).

cloned in front of a luciferase reporter gene, transiently transfected into undifferentiated Tera2 cells, and assayed for promoter activity (Fig. 2). The luciferase activity of the complete intron 12 promoter sequence of approximately 2.5 kb (clone −2500/+182) was comparable with that of the much smaller clone −668/+182, indicating that no important expression information is pertained upstream from nucleotide −668, up to exon 12 of the PDGFαR gene. A further deletion, down to position −102 (clone −102/+182), displayed only fractionally lower activity than the clones −2500/+182 and −668/+182, which further limits the region necessary for control of high level expression. The reverse orientation of the −668/+182 fragment in the pSLA4 vector (clone −668/+182R) almost completely abolished activity, demonstrating the orientation dependence of the P2 promoter. These data show that intron 12 of the PDGFαR gene contains a bona fide promoter.

Deletion mutants generated at the 3′ end (clones: −668/+14; −102/+14), which still included an intact transcription initiation site, reduced activity 4–5 times, compared with the parental clones −668/+182 and −102/+182. Thus, a cis-element determining high promoter activity is located in the transcribed part, within the region +14 to +182. The consensus octamer motif ATGCTAAT at position +28 to +35, which is present in all the constructs that show high promoter activity, was thereupon mutated to the sequence ACGCCAAT (clones −668/+182M and −102/+182M, respectively). This mutation

[3] H. J. Kraft, unpublished results.

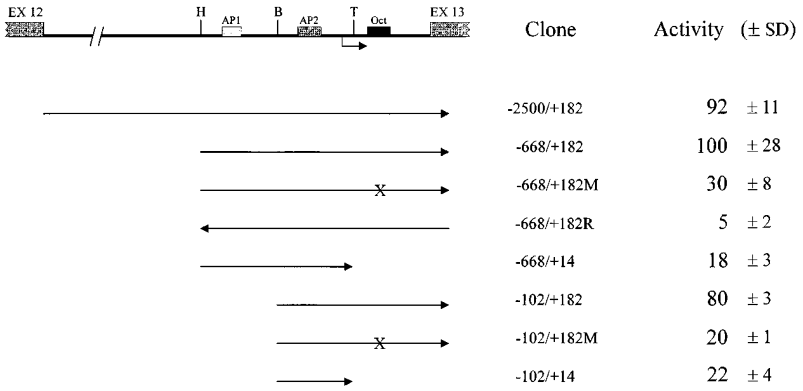

FIG. 2. **Activity of PDGFαR gene P2 promoter mutants in Tera2 EC cells is dependent on an octamer motif.** A series of 5′ or 3′ deletion mutants or of octamer motif point mutants was cloned in front of a luciferase reporter gene and transiently transfected into Tera2 EC cells. Exons (*Ex*) and restriction sites (*B*, *Bam*HI; *H*, *Hin*DIII; *T*, *Taq*I) are depicted in the figure. Luciferase activity was assayed 48 h post-transfection. Transfection efficiency was normalized for with β-galactosidase activity of a cotransfected pCH110 plasmid (Pharmacia Biotech Inc.). Values are presented as mean promoter activity relative to the clone −668/+182, which was arbitrarily set at 100% (S.D. is indicated).

is expected to abrogate all fortuitous binding of POU domain-specific proteins (24, 25). Upon changing these two nucleotides in the octamer motif, promoter activity drops by a factor of 3–4, comparable with deletion of the complete +14/+182 region (Fig. 2). This indicates that the octamer motif is indeed involved in directing P2 promoter activity in Tera2 EC cells.

In order to demonstrate that Tera2 EC nuclear proteins can actually bind to the octamer motif, an EMSA was performed. It is shown in Fig. 3*A* that the intact −102/+182 promoter fragment forms a complex with nuclear extracts of Tera2 EC cells. The formation of this complex can be specifically competed by excess (100 times) unlabeled probe itself, but not by the −102/+182M fragment containing the mutated octamer motif (*lane 4*). Moreover, the −102/+182M fragment does not form a complex in this EMSA (Fig. 3*A*, *lanes 5–8*). In addition, the −102/+182 fragment competed efficiently the four specific complexes of a consensus oct-1c oligonucleotide (Promega) with nuclear extracts of EC cells, while the −102/+182M fragment was refractory to competition (Fig. 3*B*).

In conclusion, an octamer motif is involved in the regulation of the P2 promoter of the PDGFαR gene in undifferentiated human embryonal carcinoma cells.

Oct-4 Binds to the PDGFαR P2 Promoter Octamer Motif—As described previously, the major change in constituents of octamer binding proteins during retinoic acid-induced differentiation of murine embryonal carcinoma cells involves the down-regulation of Oct-4 expression (26). We hence hypothesized that also in the undifferentiated human embryonal carcinoma Tera2 cells the POU domain transcription factor Oct-4 is present and occupies the promoter P2 octamer motif, oct-P.

In order to test this hypothesis a series of EMSAs were performed with a double-stranded oligonucleotide (oct-P), which results in a higher resolution compared with the long promoter fragment. The oct-P contains the octamer sequence and flanking 7 nucleotides at the 3′ side and 8 nucleotides at the 5′ side of the P2 promoter (+20/+42). The resulting complexes were compared with the complexes formed by the consensus oct-1c oligonucleotide (see above). The oct-P as well as the oct-1c oligonucleotide gave rise to the formation of several complexes with nuclear proteins of Tera2 EC cells, Tera2 RA cells, or mouse F9 EC cells, which could be specifically competed by excess of the respective cold probe itself (Fig. 4; see also Fig. 5). In the EMSAs, oct-1c and oct-P displayed identical bandshift patterns (not shown). Based upon the complexes formed with the nuclear extracts of mouse F9 EC cells (27, 28) a positive identification of the Oct-4 complex with Tera2 EC or RA extracts was made possible, and is indicated in Fig. 4*A*. This shows that also during RA-induced differentiation of human Tera2 embryonal carcinoma cells the POU domain transcription factor Oct-4 is down-regulated.

To confirm the results obtained with the above described EMSAs, a Northern blot analysis was performed on mRNA of Tera2 EC and Tera2 RA cells. The blot was probed with a labeled Oct-4 cDNA, which showed that Oct-4 mRNA is present in Tera2 EC cells, contrasting with the absence of any detectable Oct-4 mRNA in Tera2 RA cells (Fig. 4*B*). Conclusive evidence that the indicated Oct-4 complex in the EMSAs is formed with this transcription factor comes from a supershift analysis. This analysis was performed with an anti-Oct4-antibody (22), which only supershifted the Oct-4-containing complex and not the Oct-1 complex (Fig. 4*C*).

Hence, undifferentiated human Tera2 embryonal carcinoma cells express Oct-4, which can complex with the P2-octamer motif. The cells cease to express Oct-4 upon RA-induced differentiation, which necessarily excludes complex formation between Oct-4 and the oct-P motif.

Comparison of the oct-P Binding Site with the Consensus oct-1c Motif—The members of the POU transcription factor family are defined by their ability to bind to the octamer motif. This causes experimental pitfalls to distinguish between the binding of the different members to a particular motif in a specific cell type (29). In addition to the octamer consensus motif, however, the nucleotides juxtaposed to the motif are also important to the affinity and specificity of binding of a given Oct protein (24).[4] Therefore, we set out to compare the binding properties of the consensus oct-1c and the PDGFαR gene-derived oct-P motifs with nuclear extracts from Tera2 EC cells in EMSAs. The oct-P-derived complexes could not be competed by a 500-fold excess of the consensus oct-1c oligonucleotide, while competition with oct-P itself was easily established. A 100-fold excess of oct-P competitor was sufficient for strong competition. No signal was detectable with a 500-fold excess of competitor, even upon prolonged exposure (Fig. 5). This indicates that the oct-P oligonucleotide is bound stronger by POU proteins from Tera2 cells than the consensus oct-1c oligonucleotide. The results with the oct-P oligonucleotide are confirmed by the reciprocal experiment, using the oct-1c oligonucleotide as probe. Even with 100-fold excess, the oct-P competitor abolished the specific binding to the oct-1c oligonucleotide, while the oct-1c oligonucleotide was needed in larger

[4] H. R. Schöler, personal communication.

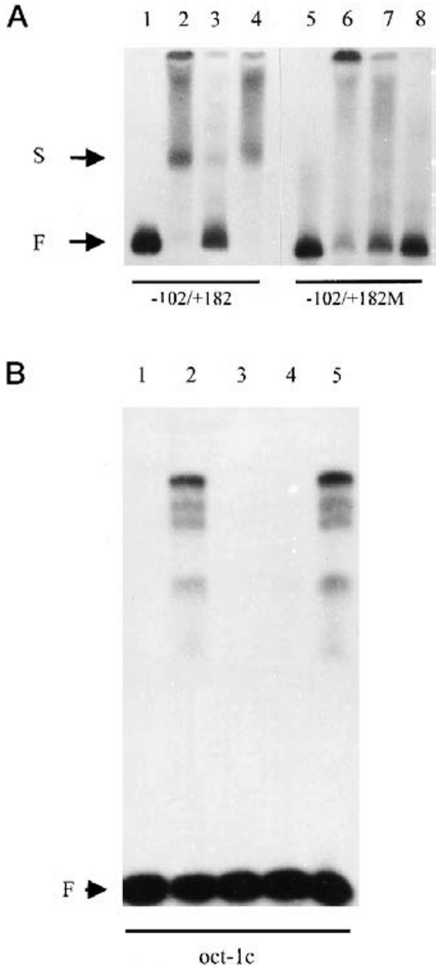

FIG. 3. **Nuclear extracts from Tera2 EC cells complex with PDGFαR P2 promoter fragments or a consensus oct-1c oligonucleotide in an EMSA.** *A*, the PDGFαR P2 promoter fragment containing the intact (probe −102/+182, *lanes 1–4*) or mutated (probe −102/+182M, *lanes 5–8*) octamer binding site was used as probe in an EMSA with nuclear extracts of Tera2 EC cells. Protein·DNA complexes were resolved on a 4.5% nondenaturing polyacrylamide gel and exposed for autoradiography for 3 days at −80 °C with intensifying screens. *Lanes 1* and *5*, free probes; *lanes 2* and *6*, probes with nuclear extract; *lanes 3* and *7*, specific competition with 100-fold excess of unlabeled fragment; *lanes 4* and *8*, nonspecific competition with 100-fold excess of unlabeled probe, mutated −102/+182M fragment, or intact -102/+182 fragment, respectively. (*S*, specific complex; *F*, free probe). *B*, a consensus oct-1c oligonucleotide (Pharmacia) was used as probe in an EMSA performed under the conditions indicated under *A*. *Lane 1*, free probe; *lane 2*, probe complexed with nuclear extract of Tera2 EC cells; *lane 3*, specific competition with 100-fold excess of unlabeled probe; *lane 4*, specific competition with 100-fold molar excess of the intact PDGFαR P2 promoter fragment −102/+182; *lane 5*, competition with 100-fold molar excess of the mutated PDGFαR P2 promoter fragment −102/+182M. (*S*, specific complex; *F*, free probe).

amounts than oct-P to compete efficiently with the labeled oct-1c·POU protein complex (not shown). Thus, the oct-P domain and flanking sequences suffice to bind Tera2 POU protein more strongly than the consensus oct-1c oligonucleotide.

Oct-4 Enhances Expression by the PDGFαR P2 Promoter in Tera2 RA Cells—The oct-P domain is a necessary determinant for high level transcription activity of the PDGFαR gene P2 promoter in undifferentiated Tera2 cells. These Tera2 EC cells endogenously contain the Oct-4 transcription factor. The level of Oct-4 decreases rapidly during RA-induced differentiation. This event coincides with ceasing amounts of promoter P2-initiated PDGFαR transcripts of 1.5 and 5.0 kb (11).[2] This suggests once again that Oct-4 acts on the oct-P domain. Hence, to investigate this presumptive role of the oct-P domain in transcription efficiency, the effect of ectopic Oct-4 expression was tested on P2 promoter luciferase constructs in Oct-4 deprived Tera2 cells.

Tera2 cells were differentiated by retinoic acid for 7 days and cotransfected with an intact or a mutated promoter construct together with a CMV-Oct-4 expression vector (Fig. 6). The activity of either intact promoter construct (−668/+182 and −102/+182, only −102/+182 is shown) was enhanced 4-fold when compared with the mock-transfected clones. Moreover, the expression levels of the mutated promoter fragment (−102/+182M) remained approximately the same, as expected. Notably, Tera2 RA cells are able to sustain a basal expression level of P2 promoter constructs. In conclusion, Oct-4 is able to bind to the oct-P domain in the PDGFαR gene P2 promoter and effectuates a 4-fold induction of expression level in cells lacking endogenous Oct-4.

DISCUSSION

We have examined the regulation of the human PDGFαR P2 promoter, which gives rise to two transcripts in Tera2 EC cells, in order to assess its role in human development and differentiation. In contrast to the P1 promoter-controlled PDGFαR transcripts, which are exclusively expressed in RA-induced differentiated cells, expression controlled by the P2 promoter is strictly confined to undifferentiated Tera2 EC cells. We have shown that intron 12 of the PDGFαR gene contains a functional promoter (P2), able to generate these alternative PDGFαR transcripts. Intron 12 of the PDGFαR gene is extremely large, approximately 2.5 kb, when compared with the structurally related proto-oncogenes c-*fms* and c-*kit*, of which the corresponding introns have lengths of 280 and 127 base pairs, respectively (11, 30, 31). The size of intron 12 of the PDGFαR gene may very well be related to the fact that it harbors a promoter. The present study clearly demonstrates that activity of the PDGFαR P2 promoter depends critically on the POU domain transcription factor Oct-4, which binds to the consensus octamer motif in the promoter region. Mutating this octamer motif resulted in a decrease of promoter activity. Moreover, in Tera2 RA cells, which contain no endogenous Oct-4, P2 promoter activity was restored by ectopic expression of Oct-4. In promoter P2 several consensus binding sites for other transcription factors are located, among others for PEA3, AP1, and AP2, which are all known to play a role in the differentiation of cells. Promoter deletion studies excluded the possibility that the binding sites for PEA3, AP1, or AP2 are involved in high level activation of promoter P2.

Considering the expression pattern of Oct-4 in *e.g.* cells of the murine inner cell mass and primordial germ cells, this transcription factor is assumed to be a key regulator in mammalian embryogenesis and is associated with an undifferentiated phenotype (24). Also, in several mouse embryonal carcinoma cell lines, *e.g.* F9 and P19, Oct-4 is expressed, and its expression is down-regulated upon differentiation. We show here that the embryonal carcinoma cell line Tera2, which is ultimately derived from a primordial germ cell, expresses Oct-4 in a differentiation dependent fashion. Moreover, Oct-4 is regarded a primordial germ cell marker, and its expression is down-regulated upon further differentiation in the testis but maintained in the oocyte (28). In this respect it is noteworthy that normal testis does not contain the 1.5-kb PDGFαR transcript.[2] This observation makes it unlikely that PDGFαR expression is regulated by Oct-6, since the POU domain transcription factor Oct-6 is expressed in the testis (28). It is presently still unclear whether Oct-4 is sufficient in P2-initiated transcription, but so far a strong correlation has been observed between promoter P2 activity and Oct-4 expression. Developmentally regulated transcriptional activation of the P2 promoter may still be depend-

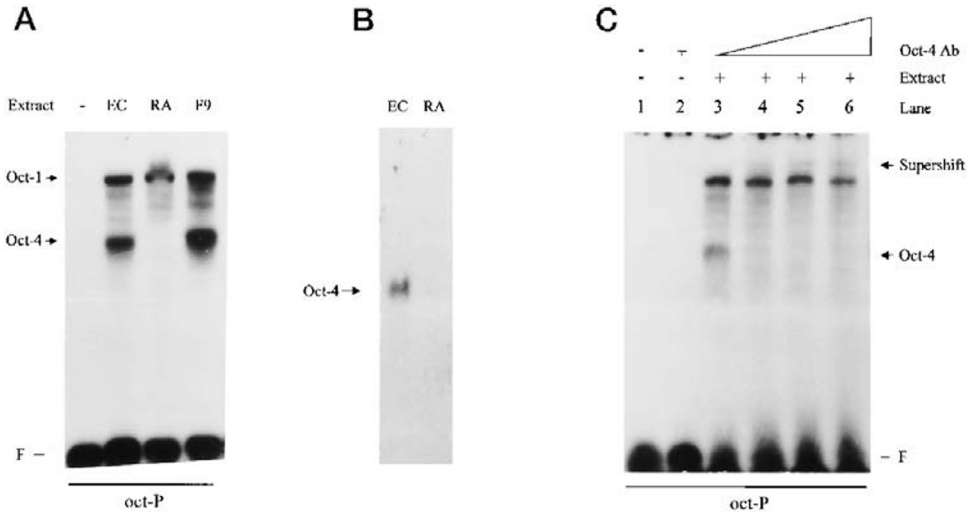

FIG. 4. **Transcription factor Oct-4 mRNA and protein is present in undifferentiated EC but not in RA-differentiated Tera2 cells.** *A*, an EMSA was performed with nuclear extracts from undifferentiated (*EC*) or 7 days RA-differentiated (*RA*) Tera2 cells or undifferentiated mouse F9 embryonal carcinoma cells (*F9*) with the PDGFαR oct-P oligonucleotide. The EMSA was performed under the conditions indicated in the legend of Fig. 3. The identified complexes are depicted in the figure with *arrows*. *F*, free probe. *B*, Northern blot analysis with mRNA of undifferentiated EC or 7 days RA-differentiated Tera2 cells. The blot was hybridized with a mouse Oct-4 cDNA probe, washed at 55 °C in 0.1 × SSC and exposed for 2 days at −80 °C with intensifying screens. *C*, supershift analysis with a mouse anti-Oct-4-antibody (see Ref. 22). The PDGFαR promoter oligonucleotide oct-P was used in an EMSA with nuclear extracts of Tera2 EC cells. A mouse anti-Oct-4-antibody was used with increasing concentrations to perform a supershift. The Oct-4 and the supershifted complexes are indicated in the figure. Conditions are as described in the legend of Fig. 3. *F*, free probe.

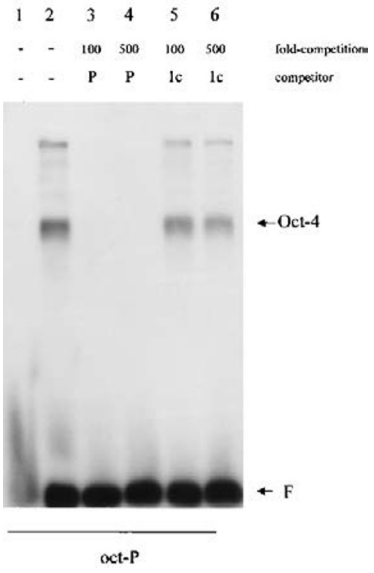

FIG. 5. **The octamer sequence of the PDGFαR P2 promoter (oct-P) displays higher binding affinity toward POU domain proteins than the consensus oct-1c site.** An EMSA was performed with nuclear extracts from Tera2 EC cells (*lanes 2–6*) and the PDGFαR oct-P oligonucleotide as probe (*F*, free probe). The complexes were competed with different-fold excess of either the cold oct-P probe (*P*) or consensus oct-1c oligo (*1c*), as indicated.

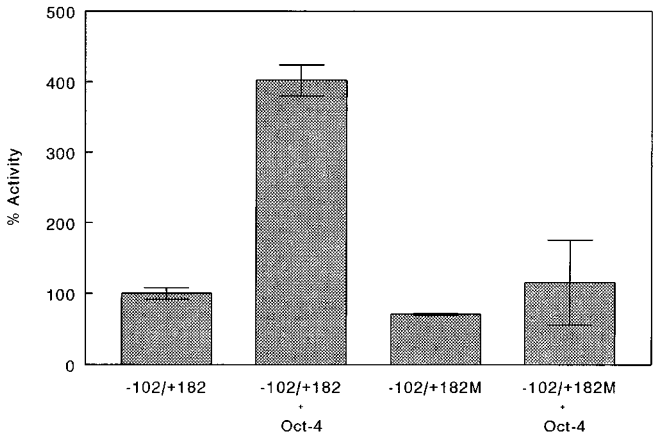

FIG. 6. **Activity of PDGFαR gene P2 promoter mutants in Tera2 RA cells is dependent on the Oct-4 transcription factor.** In the presence or absence of a cotransfected CMV-Oct-4 expression vector, the promoter clone −102/+182 or −102/+182M was transiently transfected into 7 days RA-differentiated Tera2 cells, in which Oct-4 is down-regulated. Luciferase activity was assayed 72 h post-transfection. Transfection efficiency was normalized by measuring β-galactosidase activity of a cotransfected pCH110 plasmid (Pharmacia). Values are presented as mean promoter activity relative to the clone −102/+182 without expression vector CMV-Oct-4, which was set at 100% (*error bars* indicate S.D.).

ent on auxillary proteins.

A similar mechanism has been suggested for kFGF, another effector gene of Oct-4 and a known mesoderm inducer in embryonic development. A concomitant silencing is observed in RA-induced differentiated murine EC and embryonic stem cells of both the expression of Oct-4 and kFGF. For kFGF expression, octamer-binding proteins are necessary for transcriptional activation, but developmentally restricted expression is determined by the interaction of octamer-binding proteins with Sox2 (32). The other described effector gene of Oct-4 is *REX-1*, which encodes a zinc finger-containing protein that is expressed in EC and embryonic stem cells. In mouse, RA-induced differentiation of the EC cells reduces transcription of the gene (33), which is probably due to a decline in the level of Oct-4 (22). Apart from Oct-4-controlled expression, *REX-1*, kFGF, and alternative PDGFαR transcripts contain no obvious denominator. This stresses the role of Oct-4 to stir diverse actions in development.

Oct-4, as well as any other POU domain transcription factor, is able to complex (*in vitro*) with the consensus octamer binding motif of the P2 promoter. The absence of P2-initiated messengers in RA-induced differentiated Tera2 cells or in normal placenta[2] indicates that this motif is not used promiscuously by other POU domain transcription factors like *e.g.* Oct-1, and that the specificity of binding is probably highly influenced by the flanking sequences of the motif. A preliminary comparison

of the octamer flanking sequences of kFGF, *REX-1*, and PDGFαR shows, however, that the motifs cannot be aligned adequately to explain the preferential binding of Oct-4 to these sites. Accessory proteins might therefore explain Oct-4-dependent regulation (see above).

The role of proteins encoded by these alternative PDGFαR transcripts, if any, remains obscure, since presently none of these proteins have been detected *in vivo*. The sequence of the 5.0-kb messenger suggests a putative oncogene-like action, which may be important for autonomous growth of Tera2 EC cells. Undifferentiated Tera2 cells have been shown to proliferate in the absence of serum growth factors (34). A possible role in development or differentiation may also be inferred from the expression patterns of alternative PDGFαR transcripts. P2-initiated transcripts have been identified in human oocytes and preimplantation stages[5] and in human testis tumors.[2] The down-regulation of the 1.5-kb transcript expression in spermatogenesis suggests that the P2-promoter is active in cells of the female primordial germ cell lineage, probably in the commitment of cells during embryonal development. Alternative transcripts, generated by differential promoter use and/or splicing, of other tyrosine kinase receptors have been described, including the genes for PDGFβR (4), FGF receptors (35), epidermal growth factor receptor (36), c-*kit* (37) and PDGFαR of the mouse (38). Interestingly, an alternative transcript of approximately 4.8 kb of the PDGFαR gene has been detected in the mouse embryonal carcinoma cell line F9, but only after RA-induced differentiation (38). This transcript has also been described by Lee *et al.* (39) and is regulated in a differentiation-specific manner. Any conclusive evidence concerning functions of the corresponding alternative proteins remains lacking, however.

In conclusion, the POU transcription factor Oct-4 controls the developmentally regulated expression by the PDGFαR P2 promoter. Whether the down-regulation of Oct-4 during RA-induced differentiation is a prerequisite for the activation of the PDGFαR promoter P1 remains to be determined. We are currently working toward this goal.

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